# Looking Into the Crystal Ball: A Novel Biomarker for Outcomes of Patients With Chronic Hepatitis B Virus Infection

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Patients with hepatitis B virus (HBV) infection are at an increased risk of developing liver cirrhosis and hepatocellular carcinoma (HCC), resulting in over one million deaths per year.[1] To date, there is no cure for HBV, and prolonged suppression of viral replication by inhibiting reverse transcriptase by nucleos(t)ide analogue (NA) treatment is the only treatment to reduce HCC risk.[1] However, it remains challenging to predict the residual HCC risk in patients receiving prolonged NA therapy.

Host and viral factors have been shown as important HCC risk factors in untreated patients. In patients with antiviral therapy, surrogate markers for liver fibrosis are the only risk factors in addition to patients' age and sex. In this issue, Hosaka et al.[2] report that pretreatment hepatitis B core-related antigen (HBcrAg) level, but not hepatitis B surface antigen (HBsAg) level, in serum was associated with HCC development in 180 patients negative for hepatitis B e antigen (HBeAg) who were receiving entecavir for >1 year. Patients with baseline HBcrAg level ≥10,000 U/mL (≥4.0 log U/mL) were associated with an increased HCC risk compared to those with HBcrAg below this level. A reduction of HCC risk was also observed in patients who had a decrease in HBcrAg level ≤2.9 log U/mL at 1 year after treatment.

One of the unique aspects of this particular study is that HBcrAg data were determined using the "immunoassay for total antigen including complex by pretreatment (iTACT)" technology, an ultrasensitive assay with a lower detection limit of 2.1 log U/mL compared to 3.0 log U/mL for the conventional assay. The iTACT-HBcrAg data showed that HBcrAg levels after 1 year of treatment were detectable in 92.8% of patients in contrast to 59.5% of patients using the conventional HBcrAg assay. The data suggest that transcriptionally active covalently closed circular DNA (cccDNA) remains in liver even after treatment.

A more sensitive assay is also helpful in identifying a lower cutoff to split the HCC risk. According to the iTACT-HBcrAg data, the HCC risk did not differ between patients with HBcrAg 3.0-3.9 log U/mL and those with HBcrAg 2.0-2.9 log U/mL; both risks were lower than that of patients with HBcrAg ≥4.0 log U/mL. The optimal cutoff in this on-treated cohort was set to 10,000 U/mL, which was within the quantification range of the conventional HBcrAg assay. Notably, this cutoff was the same as the cutoff used to stratify the HCC/cirrhosis risk in untreated patients who were HBeAg negative with intermediate viral load (2,000 to 20,000 IU/mL) from a Taiwanese cohort.[3,4] In addition, this cutoff has been validated in defining Caucasian patients who are HBeAg negative with mild disease by studying the cccDNA and viral RNA in liver tissue.[5]

*Abbreviations: cccDNA, covalently closed circular DNA; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; iTACT, immunoassay for total antigen including complex by pretreatment; NA, nucleos(t)ide analogue; p22cr, 22-kDa precore protein.*

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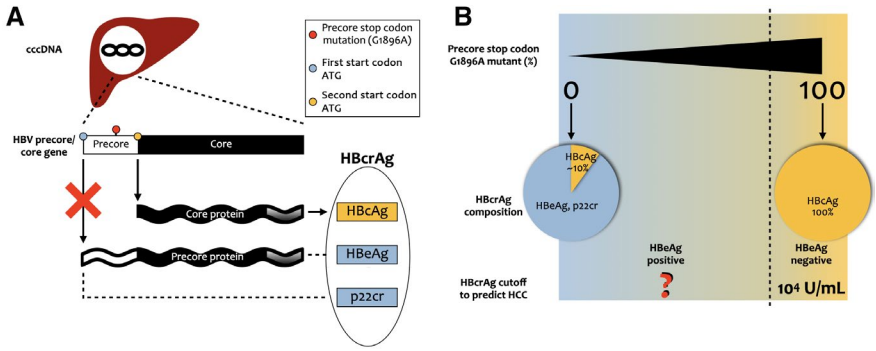


**FIG. 1.** Serum HBcrAg level is a biomarker for transcriptional activity of intrahepatic cccDNA. (A) HBcrAg is composed of HBeAg, p22cr, and HBcAg. The first two viral proteins are transcribed from the first start codon, which is affected by emergence of the precore stop codon mutation (G1896A); HBcAg is transcribed from the second start codon, which is not affected by the viral mutant. (B) The optimal HBcrAg level in defining the transcriptional activity of cccDNA may be very different between patients who are HBeAg positive and those who are HBeAg negative due to the different proportions of the precore stop codon mutation (G1896A). Abbreviation: HBcAg, hepatitis B core antigen.

Drawing together multiple lines of evidence, we believe an HBcrAg level of 10,000 U/mL is a useful cutoff to define suppressed transcriptional activity of cccDNA, which has been shown to be associated with a lower risk of disease progression and HCC development in patients who are HBeAg negative.

HBcrAg is composed of HBeAg, 22-kDa precore protein (p22cr), and hepatitis B core antigen, and the level is viewed as a serum marker of transcriptional activity of intrahepatic cccDNA.[6] However, its level could also be affected by viral mutants. Specifically, HBeAg and p22cr are about 90% of the HBcrAg, and their production could be heavily affected by emergence of the precore stop codon mutation (G1896A), which abolishes the production of both proteins (Fig. 1A).[7] As there are different proportions of precore stop codon mutation (G1896A) between patients who are HBeAg positive and those who are HBeAg negative, the serum HBcrAg cutoff to define the transcriptional activity of cccDNA could be very different between the two groups (Fig. 1B). More studies focusing on patients who are HBeAg positive should be conducted to identify the HBcrAg cutoff in predicting disease progression or HBeAg seroconversion in patients who are HBeAg positive.

A similar role of HBcrAg has also been reported in two large retrospective cohorts enrolling more than 1,000 patients who were NA treated. One cohort is from the same Japanese group that showed HBcrAg levels at 1-year posttreatment predicted HCC development.[8] The other cohort is from Hong Kong showing that an undetectable HBcrAg level (≤2.9 log U/mL) after treatment (not at a specific time point) was associated with lower HCC risk.[9] Both studies included heterogeneous populations (patients either positive or negative for HBeAg as well as those with and without liver cirrhosis). However, these results only partially support the findings from Hosaka and colleagues who enrolled only patients who were HBeAg negative. Large-scale studies with different ethnicity are warranted to validate the conclusion.

Taken together, HBcrAg is a novel hepatitis B viral marker associated with HCC development. The risk has been shown to increase when HBcrAg is >10,000 U/mL in patients who are HBeAg negative with or without NA treatment. The more sensitive assay iTACT-HBcrAg will help us decipher the kinetics in patients with low HBcrAg, which plays a key role in predicting HCC development more precisely.

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